

# Triploid individuals sired by diploid males in the polygynous ant, *Lasius sakagami*.

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## Summary

Triploid workers were born from the queens that had been inseminated by diploid males in the laboratory in the polygynous ant, *Lasius sakagami*. They were greater than diploid workers in average body size. However, no morphological difference was detected between them. The triploid workers engaged in colony tasks in the same manner as diploid workers. Furthermore, a triploid male was obtained from a field nest. The chromosomal preparation of the testis revealed no reduction of chromosome numbers throughout maturation division.

**Key words:** diploid males, spermatogenesis, triploid individuals, triploid sperm

## Introduction

To date, diploid males have been reported from more than 18 ant species (Crozier and Pamilo, 1996; Yamauchi et al. 2001). They seem to be fertile at least in a few species, judging from the facts that triploid or tetraploid individuals have been found (Crozier and Pamilo, 1996; Krieger et al., 1999; Yamauchi et al., 2001). However, few experimental studies on their sexual abilities have been made in ants. In the present study, triploid workers were born from the queens that had been inseminated by diploid males in the artificial nests in the polygynous formicine ant, *Lasius sakagami*. Furthermore, a triploid male was obtained from a field colony. We describe some characteristics of triploid individuals of this species.

## Material and Methods

Male and queen pupae with adult workers were collected from a colony inhabiting sandy herbaceous ground along the Nagara River, Gifu, Japan on July 12, 2000. After examination of ploidy for 41 males (the ratio of haploids, diploids and triploids was 46.3, 51.2 and 2.4%, respectively), the following treatments were conducted. Treatment 1: 8 queen pupae and 20 larger male pupae (presumed as diploid male) were reared with ca. 100 workers in a Petri dish (22cm diameter and 5 cm deep). After the emerged sexuals mated in the Petri dish, dealated queens were individually reared with 10 workers in a Petri dish (8.5 cm diameter and 6 cm deep, with plaster floor) by giving sugar solution and fresh pieces of mealworm. Treatment 2: 100 randomly chosen male pupae and 10 queen pupae were reared with ca. 300 workers in the larger Petri dish. Dealate queens were separately reared without workers and foods in the smaller Petri dish until the first worker emerged. Then, they were given sugar sol. and fresh mealworm pieces. All treatments were done at room temperature. 2-6 newly born workers in each of the established

colonies were examined at the prepupal stage to determine their ploidy. For chromosomal preparations, we applied an improved air-drying technique (Imai et al., 1988), using the brain of worker prepupae and the testes of pink-eye white male pupae. In addition to karyotype analysis, spermatid nuclear size was measured with these chromosomal preparations. We observed worker behavior in the established colonies, and measured head and pronotum widths of workers after fixation in 100% ethanol under a binocular microscope.

## Results

### Triploid workers

We obtained 3 established colonies in Treatment 1. 2 colonies produced triploid workers and one produced diploid workers. In Treatment 2, 8 queens laid eggs, but 6 queens brought up workers until Feb. 1, 2001. One of the 6 established colonies produced triploid workers and the rest produced diploid workers. Fig. 1A shows the chromosomes at the metaphase of a triploid worker. The haploid and diploid numbers are 15 and 30, respectively in this species (Yamauchi et al., 2001). The karyotype of triploid worker consisted of 42 acrocentric and 3 metacentric chromosomes. The triploid workers engaged in colony tasks such as foraging and taking care of the queen and larvae. In Treatment 2, the triploid worker colony and diploid worker colonies contained 18 and 6-35 workers, respectively on Feb. 1, 2001. The results of the measurements revealed that triploid workers were significantly greater than diploid workers in head width ( $t$ -test,  $p < 0.05$ ), but not significant in pronotum width. The head width (mm) was  $0.70 \pm 0.05$  (s.d.) in triploids ( $n=12$ ) and  $0.65 \pm 0.04$  (s.d.) in diploids ( $n=12$ ); the pronotum width (mm) was  $0.47 \pm 0.04$  (s.d.) in triploids ( $n=12$ ) and  $0.44 \pm 0.04$  (s.d.) in diploids ( $n=12$ ).

### Spermatogenesis of triploid male

Fig. 1B shows the chromosomes of a triploid male at the metaphase. Fig. 1C contains the chromosomes at the anaphase. The chromosomal number is 90 at the beginning of the anaphase in this individual. Then, they are divided into halves. Thus, no reduction of chromosomal numbers

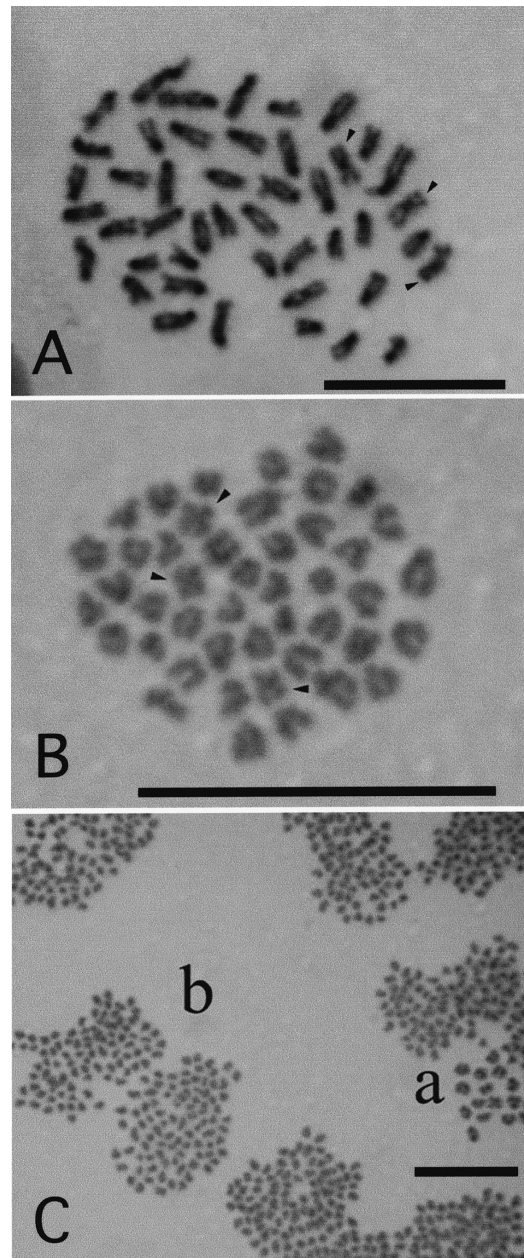


Fig.1. Chromosomes of triploid individuals of *Lasius sakagami*. A: metaphase of triploid worker; B: metaphase of triploid male; C: metaphase and anaphase of triploid male. a: metaphase; b: anaphase. Arrow: metacentric chromosomes. Scale:  $10 \mu\text{m}$ .

occurred throughout the maturation division. The spermatid nuclear size of triploid male was significantly greater than those of haploids and diploids (ANOVA,  $p < 0.0001$ ; Fisher's PLSD,  $p < 0.0001$ ). The mean maximum diameter of nuclei was  $3.1 \pm 0.1$  (s.d.)  $\mu\text{m}$  in haploids ( $n=100$ ) and  $4.2 \pm 0.2$  (s.d.)  $\mu\text{m}$  in diploids ( $n=100$ ) and  $5.8 \pm 0.2$  (s.d.)  $\mu\text{m}$  in triploid ( $n=100$ ).

#### Body size of triploid male

The Head width and pronotum width of triploid male pupa was 0.96 mm and 0.92 mm, respectively. These values were obviously greater than those of haploids: the mean was  $0.78 \pm 0.06$  (s.d.) in head width ( $n=16$ ) and  $0.78 \pm 0.04$  (s.d.) in pronotum width ( $n=16$ ). They are similar to those of the largest diploid males: the mean of diploids was  $0.89 \pm 0.06$  (s.d.) in head width ( $n=19$ ) and  $0.89 \pm 0.04$  (s.d.) in pronotum width ( $n=22$ ). However, we could not find any abnormal external morphologies in the triploid male pupa.

#### Discussion

The present study revealed that at least some diploid males actually sire viable triploid workers. These workers have normal external morphology although they are greater than diploid workers in body size. They engaged in various colony tasks and contributed to the development of the colony to the population size of 18 workers in ca. 7 months, even if it had been founded by a single queen. Therefore, it seems that colonies founded by single queens that mated with diploid males may be able to become mature and produce haploid males. However, it remains to be resolved whether triploid females can grow up to become queen caste or not, or whether or not triploid queens produce viable offspring. The triploid male from a field colony seems to be also sired by diploid males. Their spermatids were triploid, although it remained unknown whether or not they metamorphosed to functional sperm. The fact that spermatid nuclear size of triploid males was greater than those of diploids may probably explain one of the reasons why triploid individuals are greater than diploids in cell size and body size not only in ants but in other animals (Forest and Carvalho, 1994; Zhang et al., 1998).

So far diploid males of ants are thought to be inviable or infertile in the majority of species, while they are partially fertile in a few species (Crozier and Pamilo, 1996; Krieger et al., 1999). Therefore, they should be a heavy load on the colony, resulting in high mortality of diploid-male-producing monogynous colonies in natural populations (Ross and Fletcher, 1985; Pamilo et al. 1994). However, in the polygynous species (form), *L. sakagamii* and the introduced polygyne population of *Solenopsis invicta* in USA, they are frequently produced (Yamauchi et al., 2001; Ross and Fletcher, 1985; Krieger et al., 1999), and at least some diploid males sire triploid offspring. As the triploid workers engage in colony tasks in *L. sakagamii* and probably in *S. invicta*, it seems that the load on the colony caused by diploid male production may be not so heavy as has been previously thought.

#### Acknowledgements

This study was supported in part by a Grant-in-Aid for Scientific Research (No. 12640612) from the Japan Ministry of Education, Culture, Sports, Science and Technology.

## References

- Crozier, R. H. and P. Pamilo, 1996. *Evolution of Social Insect Colonies. Sex Allocation and Kin Selection*. Oxford University Press, Oxford, New York, Tokyo, 306p.
- Forest, F. and C. Carvalho, 1994. Ploidy evaluation in the pacu fish, *Piaractus mesopotamicus* (Pisces, Characiformes): Techniques and comments. *Revista Brasileira de Biologia* 54: 31-37.
- Imai, H.T., R.W. Taylor, M.W.J. Crosland and R. H. Crozier, 1988. Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis. *Jpn. J. Genet.* 63: 159-185.
- Krieger, M.J.B., K.G. Ross, C.W.Y. Chang and L. Keller, 1999. Frequency and origin of triploidy in the fire ant *Solenopsis invicta*. *Heredity* 82: 142-150.
- Pamilo, P., L. Sundstrom, W. Fortelius and R. Rosengren, 1994. Diploid males and colony level selection in Formica ants. *Ethol. Ecol. Evol.* 6: 221-235.
- Ross, K.G. and D.J.C. Fletcher, 1985. Genetic origin of male diploidy in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), and its evolutionary significance. *Evolution* 39: 888-903.
- Yamauchi, K., T. Yoshida, T. Ogawa, S. Itoh, Y. Ogawa, S. Jimbo and H. T. Imai, 2001. Spermatogenesis of diploid males in the formicine ant, *Lasius sakagami*. *Insectes Soc.* 48: 28-32.
- Zhang, G., Z. Yang, Y. Chang, J. Song, J. Ding, Y. Wang and R. Wang, 1998. Triploid induction in Pacific abalone *Haliotis hannai* Ino by 6-dimethylaminopurine and the performance of triploid juveniles. *Journal of Shellfish Research* 17: 783-788.